

Clinical trial of hepatitis B vaccine in a simplified immunization programme

P. COURSAGET,¹ B. YVONNET,^{2, 3} M. SARR,² P. VINCELOT,¹ E. TORTEY,¹ S. MBOUP,² J. P. CHIRON,³ & I. DIOP-MAR²

The immunogenic effect on Senegalese infants of two doses of hepatitis B vaccine with a 6-month interval followed by a booster dose after another 6 months was studied. Anti-HBs antibodies were detected in 65.8% of the infants 6 months after the first injection (geometric mean titre (GMT), 6.1 mIU/ml). At the time of the booster injection, 89.7% of the infants exhibited anti-HBs antibodies (GMT, 83.7 mIU/ml). Two months after the booster dose, 95.4% of the infants were anti-HBs positive (GMT, 348 mIU/ml). This anamnestic anti-HBs response is lower than that produced by two doses of the vaccine at a 2-month interval (GMT, 670 mIU/ml) or three doses at a 1-month interval (GMT, 1500 mIU/ml).

In developing countries immunization schedules tend to mirror the practices followed in developed countries, and therefore consist of three separate sessions and a fourth booster dose. However, such a schedule is usually only possible in health centres located in the main urban areas of developing countries. In rural areas, in contrast, access to health care centres is more limited and mobile teams for immunization are required. The areas covered by these teams are large, in general, and long periods occur between sessions. For example, schedules comprising two or three injections at 6-month intervals or two injections at a 1-year interval have been used for tetanus, diphtheria, and pertussis immunizations, and similar procedures are being considered for polio immunization schedules (1-4). Clearly, a schedule involving a 6-month interval greatly facilitates the organization of immunization programmes and may give high returns in terms of disease prevention (2).

We have previously reported (5) that a schedule consisting of two doses of 5 µg HBsAg vaccine with a 2-month interval plus a booster dose 1 year later produces an immune response comparable to that obtained with the traditional protocol of three injections at a 1-month interval plus a booster 1 year

later (6). Here, we describe the results of a study carried out in rural areas of Senegal to assess the immunogenic effect of two doses of hepatitis B vaccine with a 6-month interval followed by a booster dose after another 6 months, and compare them with those obtained using two doses of vaccine with a 2-month interval or three doses at 1-month intervals (5, 7).

MATERIALS AND METHODS

Vaccine

Hepatitis B vaccine^a containing 5 µg of HBsAg was used and administered by subcutaneous injection into the upper arm.

Laboratory methods

Levels of HBsAg,^b anti-HBs,^c and anti-HBc^d were determined by radioimmunoassay. The concentration of anti-HBs antibody (in mIU/ml) was determined using the method reported by Hollinger et al. (8).

¹ Institut de Virologie de Tours, Facultés de Médecine et de Pharmacie, 2 bis boulevard Tonnellé, 37032 Tours, France. Requests for reprints should be sent to Dr P. Coursaget at this address.

² Faculté de Médecine et de Pharmacie, Dakar-Fann, Senegal.

³ Laboratoire de Microbiologie-Immunologie, Faculté de Pharmacie, Tours, France.

^a Havac B°. From Pasteur Vaccins, Marnes la Coquette, France.

^b Ausria II. From Abbott Laboratories, North Chicago, IL, USA.

^c Ausab. From Abbott Laboratories.

^d Corab. From Abbott Laboratories.

Immunization protocol

Infants received three injections of hepatitis B vaccine at 6-month intervals (T_0 , T_6 , and T_{12} , respectively), with the third dose considered as a booster. The following vaccines were also administered to subsets of children: BCG and diphtheria/tetanus/pertussis-polio (DTP-polio) at T_0 and DTP-polio at T_6 and T_{12} (9).

Study population

The study was carried out in the *département* of Fatick, Senegal. A total of 664 infants received the first dose of hepatitis B vaccine, 409 the second dose, and 177 the third. Blood samples were taken at the time of each injection (T_0 , T_6 , and T_{12}), and in the case of 89 infants also 2 months after the last (booster) dose (T_{14}). The follow-up loss was therefore high, since only 26.7% of the infants completed the entire series of injections (Table 1). Only results from infants who were seronegative at T_0 are shown, i.e., 281 infants at T_6 , 116 at T_{12} , and 65 at T_{14} . At T_0 the mean age of the seronegative infants was 10.2

months, while that of the seropositive infants with anti-HBs antibodies was 7.4 months. The mean age of infants who were only anti-HBc-positive was 4.8 months and that of infants who were already HBsAg-positive at T_0 was 14.3 months.

The results obtained were compared with those reported previously for two other groups of Senegalese infants (5, 7). The first of these comprised 72 seronegative infants who were immunized using a protocol of two doses of hepatitis B vaccine with a 2-month interval, while the second consisted of 111 seronegative infants immunized using three doses at 1-month intervals. Both groups also received a booster 12 months after the first dose.

RESULTS

The anti-HBs response was determined 6 months after the T_0 dose of hepatitis B vaccine for the 281 infants who were seronegative. Of these children, 185 (65.8%) exhibited anti-HBs antibodies but the geometric mean titre (GMT) was only 6.1 mIU/ml. The

Table 1. Some characteristics of the infants in the study population

Event	No. of infants	Percentage of infants followed up	No. of seronegative infants
First dose (T_0)	664	—	—
Second dose (T_6)	409	61.6	281 (68.8) ^a
Third dose (T_{12})	177	26.7	116 (65.5)
Two months after the booster injection (T_{14})	89	13.4	65 (73.0)

^a Figures in parentheses are percentages.

Table 2. Immune response to hepatitis B vaccine among seronegative infants who received three doses at 6-month intervals

Characteristic	Time		
	6 months after 1st dose (T_6)	6 months after 2nd dose (T_{12})	2 months after booster dose (T_{14})
No. of infants	281	116	65
No. with anti-HBs antibodies	185 (65.8) ^a	104 (89.7)	62 (95.4)
Geometric mean titre (mIU/ml)	6.1	83.7	348.0

^a Figures in parentheses are percentages.

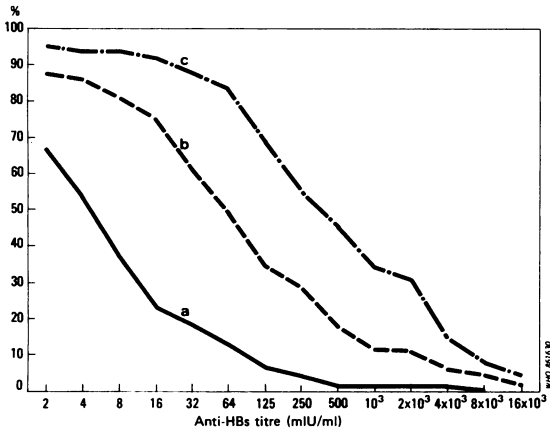


Fig. 1. Cumulative distribution of anti-HBs titres in 65 seronegative infants at various times following injection with hepatitis B vaccine: (a) six months after the first dose (T₆); (b) six months after the second dose (T₁₂); (c) two months after the booster dose (T₁₄).

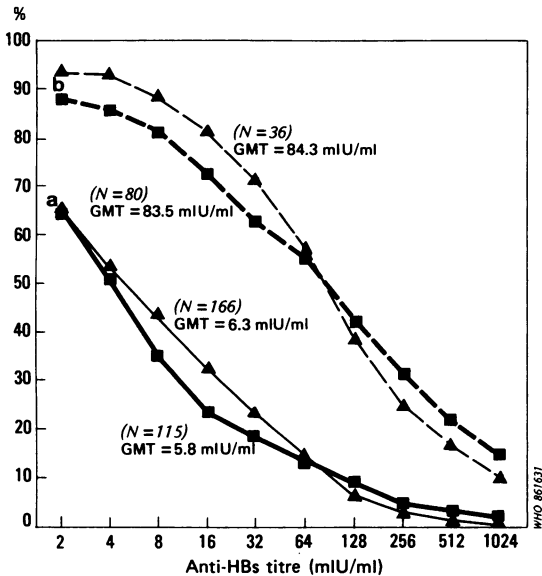


Fig. 2. Cumulative distribution of anti-HBs titres in study infants following the first two doses of hepatitis B vaccine. The plots show the results for infants who received hepatitis B vaccine only or together with diphtheria/tetanus/pertussis and polio (DTP-polio) vaccines (▲ hepatitis virus B vaccine; ■ hepatitis virus B vaccine plus DTP-polio vaccine): (a) six months after the first dose (T₆); (b) six months after the second dose (T₁₂) (GMT = geometric mean titre).

anti-HBs response of the 116 infants who received the second dose of vaccine was determined when the third (booster) injection was given (T₁₂): 104 were positive for anti-HBs (89.7%), and the anti-HBs GMT was 83.7 mIU/ml. Assay of blood samples from 65 infants 2 months after the booster dose (T₁₄) indicated that 62 (95.4%) had anti-HBs antibodies, the anti-HBs GMT reaching 348 mIU/ml (Table 2).

The geometric mean titres of anti-HBs antibodies in the 65 infants who were followed over 14 months increased from 5.6 mIU/ml (T₆) to 67.0 mIU/ml (T₁₂) and finally to 348 mIU/ml (T₁₄) (Fig. 1). The increase in titre was more important between T₆ and T₁₂ than between T₁₂ and T₁₄, which indicates that the second injection had a booster effect.

A subset of infants received concomitantly with the hepatitis B vaccine also BCG and DTP-polio vaccines at T₀ as well as DTP-polio at T₆ and T₁₂. Seroconversion at T₆ occurred in 65.7% of the 166 infants who were immunized only against hepatitis and in 65.2% of the 115 infants who also received BCG and DTP-polio vaccine; the geometric mean titres for the two sets of infants were 6.3 mIU/ml and 5.8 mIU/ml, respectively (Fig. 2).

In all, 94% of infants who received only hepatitis B vaccine seroconverted 6 months after receiving the

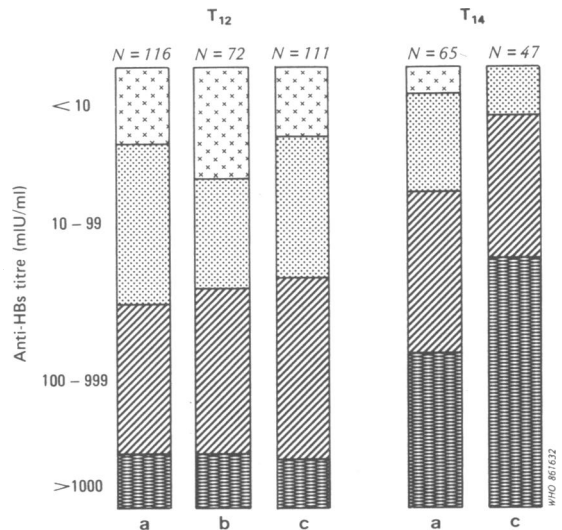


Fig. 3. Distribution of anti-HBs titres at the time of the booster dose (T₁₂) and 2 months after the booster dose (T₁₄) in infants who received one of the following vaccination protocols: (a) two doses of hepatitis virus B vaccine with a 6-month interval; (b) two doses with a 2-month interval; or (c) three doses at 1-month intervals.

Table 3. Results of follow up studies of infants who were seronegative at the initial dose of vaccine (T_0)

Characteristic	Time		
	T_0-T_6	T_6-T_{12}	$T_{12}-T_{14}$
No. of seronegative infants	281	114	63
No. of HBsAg-positive infants	5 (1.8) ^a	—	—
No. of cases of hepatitis B infection	8 (2.8)	2 (1.8)	—

^a Figures in parentheses are percentages.

second dose at T_{12} (GMT, 84.3 mIU/ml). For infants who also received BCG and DTP-polio vaccine, the corresponding rate of seroconversion was 87.5% (GMT, 83.5 mIU/ml).

The distribution of anti-HBs titres found in the present study is compared in Fig. 3 with those of 72 seronegative infants who received two doses of hepatitis B vaccine at a 2-month interval and 111 seronegative infants immunized with three doses at 1-month intervals (both sets of infants also received a booster at T_{12}): the rates of seroconversion were similar at the end of each vaccination schedule (T_{12}) (89.7%, 93.1%, and 94.6%, respectively; GMT of anti-HBs: 84, 82, and 92 mIU/ml, respectively). However, at T_{14} the geometric mean titre of the 65 infants in the present study (348 mIU/ml) was lower than that of the 47 infants who received three doses of the vaccine (1500 mIU/ml), as was also the proportion of infants with high anti-HBs titres.

Hepatitis B infection occurred in eight (2.8%) of 281 susceptible infants during the first 6 months of the study (Table 3), five of whom were HBsAg-positive. Over the second 6 months (T_6-T_{12}), 114 susceptible infants were followed up: none became HBsAg-positive, but two (1.8%) exhibited anti-HBc antibodies. No cases of hepatitis B occurred among the 63 susceptible infants studied during the 2 months following the booster injection ($T_{12}-T_{14}$).

CONCLUSIONS

The results of the study establish that infants administered two 5- μ g doses of hepatitis B vaccine with a 6-month interval exhibit a seroconversion rate and antibody levels comparable to those produced using a protocol comprising two doses with a 2-month interval or three doses at 1-month intervals.

No age-dependent anti-HBs response was observed for the 3–24-month-old infants at the time of the first injection. However, following the booster injection given 1 year after the initial dose in each protocol, the anamnestic response was lower for the two doses/6 months schedule (GMT, 348 mIU/ml) than for the two doses/2 months (GMT, 670 mIU/ml) or three doses/1 month schedule (GMT, 1500 mIU/ml). Since the level of anti-HBs decreases with time (10, 11), the booster dose should be administered earlier than 12 months after the first injection.

The level of protection afforded by the two doses/6 months schedule, as indicated by the presence of 1.8% HBsAg-carriers at the time of the second injection, is approximately half that afforded by natural infection (6, 12, 13). This implies that the presence of only a small amount of anti-HBs is protective. The vaccine used therefore contained pre-S gene products (14), which induce highly protective antibodies (15). It should be noted, however, that the protective levels of anti-HBs will vary according to the particular vaccine used.

A schedule of immunization involving three vaccinations at 6-month intervals greatly facilitates the organization of immunization programmes in the rural areas of developing countries, since each village has to be visited only twice per annum. However, a disadvantage of such a schedule is that yellow fever and measles vaccines are then administered at the same time as the hepatitis booster to 15–18-month-old infants, which is relatively late for these two vaccines.

In order to reduce the disadvantages of the 6-month protocol, an immunization schedule of three injections with a 3-month interval is being studied. This would have the advantage of reducing the period of low protection between the first and the second doses, while the booster injection (T_6) would be given to 9–12-month-old infants.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministère de la Coopération (France) and the Secrétariat d'Etat à la Recherche Scientifique et Technique (Senegal).

RÉSUMÉ

ESSAI CLINIQUE DU VACCIN ANTI-HÉPATITE B DANS LE CADRE
D'UN PROGRAMME SIMPLIFIÉ DE VACCINATION

La réponse immunitaire anti-HBs a été étudiée chez des enfants sénégalais recevant deux doses de vaccin anti-hépatite B à 6 mois d'intervalle. Une dose de rappel était injectée 6 mois après la deuxième dose de vaccin. Les résultats obtenus ont été comparés à ceux obtenus lors de deux études antérieures qui utilisaient des protocoles à 2 doses injectées à 2 mois d'intervalle et à 3 doses injectées à 1 mois d'intervalle.

Les anticorps anti-HBs ont été détectés chez 65,8% des enfants 6 mois après la première injection de vaccin (le jour de la deuxième injection). Le titre moyen géométrique des anticorps était de 6,1 mUI/ml. Le jour de l'injection de rappel, 6 mois après la deuxième injection, 89,7% des enfants étaient anti-HBs positifs et le titre moyen géométrique des anticorps était de 83,7 mUI/ml. Ces résultats

sont comparables à ceux observés avec les deux autres protocoles de vaccination. Après l'injection de rappel, 95,5% des enfants étaient anti-HBs positifs, avec un titre moyen géométrique des anticorps de 348 mUI/ml. Ce chiffre est plus faible que celui observé dans les deux autres protocoles de vaccination (670 et 1500 mUI/ml).

Les résultats montrent également que la protection est faible pendant les 6 premiers mois, c'est-à-dire avant la deuxième injection, et que la troisième injection intervient chez des enfants relativement âgés. Il est alors un peu tard pour associer cette troisième injection aux vaccins anti-amaril et antirougeoleux. Un protocole à 3 injections à 3 mois d'intervalle devrait être plus approprié aux conditions de terrain, tout en apportant une meilleure protection.

REFERENCES

1. DURAND, B. ET AL. Vaccination antitétanique simplifiée: Résultats préliminaires d'une étude africaine. *Developments in biological standardization*, 41: 3-14, (1978).
2. MANGAY-ANGARA, A. ET AL. A two-dose schedule for immunization of infants using a more concentrated DPT-vaccine. *Developments in biological standardization*, 41: 15-22 (1978).
3. FILLASTRE, C. ET AL. Clinical trial of concentrated inactivated polio vaccine in a simplified immunisation program. *Developments in biological standardization*, 47: 207-213 (1981).
4. MONTAGNON, B. J. ET AL. The large-scale cultivation of vero cells in micro-carrier culture for virus vaccine production. Preliminary results for killed poliovirus vaccine. *Developments in biological standardization*, 47: 55-64 (1981).
5. YVONNET, B. ET AL. Immunogenic effect of hepatitis B vaccine in children: comparison of two- and three-dose protocols. *Journal of medical virology*, 14: 137-139 (1984).
6. MAUPAS, P. ET AL. Efficacy of hepatitis B vaccine in prevention of early HBsAg-carrier state in children. Controlled trial in an endemic area (Senegal). *Lancet*, 1: 289-292 (1981).
7. COURSAGET, P. ET AL. Immune response to hepatitis B vaccine in infants and newborns: controlled trial in an endemic area (Senegal). In: Williams, A. O. et al., ed. *Virus-associated cancers in Africa*. (IARC Scientific Publications No. 63), Lyon, International Agency for Research on Cancer, 1984, pp. 319-335.
8. HOLLINGER, F. B. ET AL. Response to hepatitis B vaccine in a young adult population. In: Szmuness, W. et al., ed. *Viral hepatitis*. Philadelphia, PA, Franklin Institute Press, 1982, pp. 451-466.
9. COURSAGET, P. ET AL. Simultaneous administration of diphtheria/tetanus/pertussis/polio and hepatitis B vaccines in a simplified immunization program: immune response to diphtheria toxoid, tetanus toxoid, pertussis and hepatitis B surface antigen. *Infection and immunity*, 51: 784-787 (1986).
10. GOUDEAU, A. ET AL. Hepatitis B vaccine: clinical trials in high-risk settings in France. *Developments in biological standardization*, 54: 267-284 (1983).
11. JILD, W. ET AL. Hepatitis B vaccination: how long does protection last? *Lancet*, 2: 458 (1984).
12. MAUPAS, P. ET AL. Vaccination against hepatitis B in an endemic area. Prevention of the early HBsAg carrier state in children. In: Maupas, P. & Guesry, P., ed. (INSERM Symposium No. 18). Amsterdam, Elsevier/North-Holland Biomedical Press, 1981, pp. 213-224.
13. COURSAGET, P. ET AL. Hepatitis B vaccine: immunization of children and newborns in an endemic area (Senegal). *Developments in biological standardization*, 54: 245-257 (1983).
14. NEURATH, A. R. ET AL. Enzyme-linked immunoassay of pre-S gene-coded sequences in hepatitis B vaccines. *Journal of virological methods*, 12: 185-192 (1985).
15. NEURATH, A. R. ET AL. Hepatitis B virus contains pre-S gene-encoded domains. *Nature*, 315: 154-156 (1985).